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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/733,288 | 12/12/2003 | Makoto Kaibara | P24684 | 2562 |

7055 7590 11/29/2005

GREENBLUM & BERNSTEIN, P.L.C.
1950 ROLAND CLARKE PLACE
RESTON, VA 20191

| EXAMINER | |
|---|---|
| SZPERKA, MICHAEL EDWARD | |
| **ART UNIT** | **PAPER NUMBER** |
| 1644 | |

DATE MAILED: 11/29/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/733,288 | KAIBARA ET AL. | |
| | Examiner | Art Unit | |
| | Michael Szperka | 1644 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on *26 August 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-6 and 8-29* is/are pending in the application.

4a) Of the above claim(s) *8-29* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *26 August 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *8/26/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: _____.

## DETAILED ACTION

1. Applicant's reply and amendment received August 26, 2005 is acknowledged.

Claims 1, 5, 6, 10, 11, 13, 14, 17, and 20-24 have been amended.

Claim 7 has been cancelled.

Claims 25-29 have been added.

Claims 8-24 stand withdrawn for the reasons of record set forth in the office action mailed May 27, 2005.

Newly submitted claims 25-29 are directed to an invention that is independent or distinct from the invention originally claimed for the following reasons: The newly added claims are directed to methods of use, specifically administration of a factor IX activating protein to inhibit the formation of activated factor IX. The claims currently under examination, 1-6, read on a product that activates factor IX. This product can be used in other methods other than those recited in the newly presented claims, an example of such a different use being the administration of the product of claims 1-6 to a mouse for the purpose of producing monoclonal antibodies that bind said product.

Since applicant has received an action on the merits for the originally presented invention, this invention has been constructively elected by original presentation for prosecution on the merits. Accordingly, claims 25-29 are withdrawn from consideration as being directed to a non-elected invention. See 37 CFR 1.142(b) and MPEP § 821.03.

It is noted that applicant continues to traverse the restriction requirement and indicated that the claims newly added should be examined with the elected group. This has not been found convincing for the reasons given above. Applicant is reminded of the possibility of rejoinder, and is directed to see paragraph 10 of the restriction requirement mailed March 7, 2005.

Claims 1-6 are under examination in this office action.

***Information Disclosure Statement***

2. Applicant's IDS submitted August 26, 2005 is acknowledged and has been considered. It is also noted that applicant has submitted a copy of the IDS originally received March 12, 2004. All references on this copy that were indicated as considered with the office action mailed May 27, 2005 have been lined through as duplicate references. Documents 2 (Kaibara et al.) and 11 (Nakamura et al.) originally not considered have been considered only to the extent that these references are discussed in the instant specification at the passages cited by applicant on page 21 of the response received August 26, 2005 since the examiner cannot read Japanese and translations of these documents have not been provided.

***Specification***

3. Applicant is thanked for the amendments to the specification that satisfactorily correct the minor informalities that had been noted in the disclosure in the office action

mailed May 27, 2005. Applicant's cooperation is again requested in correcting any errors of which applicant may become aware in the specification.

### *Claim Objections*

4. The objections to claim 1 have been overcome based upon applicant's amendment to the claim and persuasive argument.

Claim 2 is objected to because the amino acid glycine is misspelled.

### *Claim Rejections - 35 USC § 112*

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. The rejection of claims 1-6 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement because it did not teach how to make or use the polypeptide of SEQ ID NO:4 has been withdrawn in view of applicant's amendment to the sequence listing and reasonable explanation for the unintentional error that resulted in a discrepancy between the electronically filed version of SEQ ID NO:4 and the material taught in the specification. As such, the enzyme being claimed in the instant claims contains a tyrosine (Y) at position 80, and not a threonine (T). Therefore,

the specification does teach how to make and use such an enzyme. Applicant has also entered comments in the response regarding the anticipation of the instant claims, and these arguments will be addressed later in this office action.

7. The following are new grounds or rejection necessitated by Applicant's amendments to the claims received August 26, 2005 and most specifically to applicant's amendments to the sequence listing that changes the scope of said claims that was also received August 26, 2005.

8. Claims 5 and 6 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a pharmaceutical composition comprising the polypeptide of SEQ ID NO:4 that is used to treat diseases associated abnormal blood coagulation caused by an inability to convert factor IX to factor IXa, does not reasonably provide enablement for a pharmaceutical composition comprising the polypeptide of SEQ ID NO:4 that is used for prevention of any and all blood coagulation diseases. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Applicant has claimed a pharmaceutical composition comprising a factor IX activating enzyme (i.e. the polypeptide of SEQ ID NO:4) that is to be administered to

treat and to prevent blood coagulation diseases. Hemophilia B is a blood coagulation disease that is caused by genetic defects, such missense, frameshift, and deletion mutations, in the patient's factor IX gene (Rick et al., Hematology, 2003, pages 559-574, see entire document, particularly the first full paragraph of page 567). Administration of the claimed product will not prevent hemophilia B since the instant product does nothing to repair the genetic lesion in the factor IX gene or to stop the initial formation of this genetic lesion. Indeed, hemophilia B is most commonly a congenital disorder, with the patient receiving the defective factor IX allele from a parent (MedlinePlus encyclopedia entry for hemophilia B, see entire selection downloaded 11/14/05). Therefore prevention of hemophilia B would need to occur either at, or prior to, the time when the patient is conceived, and the specification does not appear to teach such methodologies. Further, administration of the claimed pharmaceutical composition activates factor IX that is already present in the patient. Hemophilia B patients make factor IX that is incapable of normal participation in the coagulation cascade, and as such administering an enzyme to activate defective factor IX in hemophilia B patients will not fix the defective factor IX molecules and allow normal blood coagulation to occur in hemophilia B patients. As such, a skilled artisan would be unable to use the claimed product for the full scope of the recited intended use without conducting additional research.

***Claim Rejections - 35 USC § 102***

9. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

10. Claims 1-6 are rejected under 35 U.S.C. 102(b) as being anticipated by Aoki (J. Biol. Chem. 1978, 253:2026-2032, see entire document) as evidenced by Alberts et al. (Molecular Biology of the Cell, third edition, 1994, pages 1164-1167, see entire selection), as evidenced by Okano et al. (J. Biochem 1987, 102:13-16, of record on the 892 mailed May 27, 2005, see entire document) as evidenced by Iwata et al. (Biochem Biophys Res Commun. (2004) 316:65-70, of record on the 892 mailed May 27, 2005, see entire document) as evidenced by Takahashi et al. (J Biol. Chem., 1988, 263:14739-14747, or record as reference 1 on the IDS received August 26, 2005, see entire document) and as evidenced by Korkmaz et al. (J Immunol. 2005, 3329-3338, see entire document).

Aoki teaches the purification of a protease from human bone marrow cells and its presence in pharmaceutical compositions comprising physiologically acceptable buffer solutions (see entire document, particularly the abstract and the miniprint results section and miniprint Figure 3 that accompanies the article). The enzyme isolated by Aoki et al. has an apparent molecular weight of 28 kDa by SDS-PAGE, which is approximately 25.7 kDa (see the first full paragraph of the left column of page 2027). Note that bone marrow contains immature erythroblasts as well as mature erythrocytes (Alberts et al.,

see entire selection, particularly Figure 22-27 on page 1166) and that Aoki did not separate out particular cell types from bone marrow before beginning his extraction procedure (see particularly the miniprint results subsection titled Extraction in the rightmost column of page 2030). This enzyme purified by Aoki was later sequenced and given the name Medullasin (Okano et al., see entire document, particularly the first paragraph of the left column of page 13, the first full paragraph of page 15, and Figure 2). Okano et al. report that the final residue present in natural medullasin is arginine, the same final amino acid as SEQ ID NO:4 of the instant application, and that the amino acid sequences of several medullasin fragments were completely identical to the amino acid sequence deduced from the cDNA sequence (see particularly the legend to figure 2 and the first two sentences of the paragraph that spans the left and right columns of page 15). Medullasin is an alternate name for neutrophil elastase (see enclosed copy of the search printout, Result 1 in the UniProt database, which includes a listing of publications dealing with the isolation and properties of medullasin/neutrophil elastase and an alignment of this sequence with SEQ ID NO:4, which indicates that SEQ ID NO:4 is 100% contained within the sequence of Result 1). As such, the enzyme isolated by Aoki et al. ends in a carboxyl terminal arginine and is identical in sequence to neutrophil elastase, and therefore, the enzyme isolated by applicants as activating factor IX is also neutrophil elastase. Further, applicant discloses in a post filing peer-reviewed journal publication that "The amino acid sequence of EE-IX is in accord with that of neutrophil elastase" (Iwata et al., see entire document, particularly Table 1 and the first and second paragraphs of the Discussion section on page 69, and note that

EE-IX is a term used in the paper and in the instant specification that refers to the polypeptide of SEQ ID NO:4). It is also worthy of note that Takahashi et al. teach that neutrophil elastase is represented only once in the human haploid genome (see entire document, particularly the paragraph that spans pages 14743 and 14744), and as such there cannot be separate genes for medullasin/neutrophil elastase and the factor IX activating enzyme of SEQ ID NO:4 currently claimed by applicant.

It is noted that none of the aforementioned art indicates that medullasin/neutrophil elastase is inhibited by $\alpha$1-protease inhibitor or soybean trypsin inhibitor, but these properties are inherent to medullasin/neutrophil elastase, as evidenced by Korkmaz et al. who teach that $\alpha$1-protease inhibitor inhibits neutrophil elastase (see entire document, particularly the abstract).

Applicant has also recited other properties of the claimed enzyme, including the ability to cleave factor IX at a specified location. Since as has been discussed above, the sequence of the prior art enzyme and that of SEQ ID NO:4 are the same, functional activities such as the ability to cleave factor IX are inherent. Applicant is reminded that "[T]he discovery of a previously unappreciated property of a prior art composition, or of a scientific explanation for the prior art's functioning, does not render the old composition patentably new to the discoverer." Atlas Powder Co. v. Ireco Inc., 190 F.3d 1342, 1347, 51 USPQ2d 1943, 1947 (Fed. Cir. 1999). Thus the claiming of a new use, new function or unknown property which is inherently present in the prior art does not necessarily make the claim patentable. In re Best, 562 F.2d 1252, 1254, 195 USPQ 430, 433 (CCPA 1977). See also MPEP 2112.

It is further noted that claim 4 claims the instant product by a product by process relationship. Applicant is also reminded that "[E]ven though product-by-process claims are limited by and defined by the process, determination of patentability is based on the product itself. The patentability of a product does not depend on its method of production. If the product in the product-by-process claim is the same as or obvious from a product of the prior art, the claim is unpatentable even though the prior product was made by a different process." In re Thorpe, 777 F.2d 695, 698, 227 USPQ 964, 966 (Fed. Cir. 1985) (citations omitted) and MPEP 2113. Since the product of the prior art and the product of the instant claims have the same amino acid sequence, the structure of the instant claimed product is that of the prior art and thus the manner by which Aoki isolated medullasin/neutrophil elastase is not relevant to patentability.

Applicant has indicated on pages 24 and 25 of the reply received August 26, 2005 arguments as to why prior art documents discussed as part of an enablement rejection in the office action mailed May 27, 2005 would not be relevant as anticipatory prior art. The argument is that the prior art references, some of which are discussed in the instant rejection, do not account for all of the properties of the claimed product recited in instant claim 1. Such arguments have been considered but are not persuasive for the reasons discussed above.

Therefore, the prior art anticipates the claimed invention.

11. No claims are allowable.

12. Applicant's amendment necessitated the new grounds of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Szperka whose telephone number is 571-272-2934. The examiner can normally be reached on M-F 8-4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on 571-272-0841. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Michael Szperka, Ph.D.
Patent Examiner
Technology Center 1600
November 14, 2005

Patrick J. Nolan, Ph.D.
Primary Examiner
Technology Center 1600

GenCore version 5.1.6


OM protein - protein search, using sw model

Run on: October 24, 2005, 13:10:07 ; Search time 170 Seconds
(without alignments)
659.678 Million cell updates/sec

Title: US-10-733-288B-4
Perfect score: 1142
Sequence: 1 IVGGRRARPHAWPFMVSLQL.....PDAFAPVAQFVNWIDSIIQR 219

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1612378 seqs, 512079187 residues

Total number of hits satisfying chosen parameters: 1612378

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : UniProt_03:*
1: uniprot_sprot:*
2: uniprot_trembl:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

| Result No. | Score | % Query Match | Length | DB | ID | Description |
|---|---|---|---|---|---|---|
| 1 | 1142 | 100.0 | 267 | 1 | ELNE_HUMAN | P08246 homo sapien |
| 2 | 905 | 79.2 | 282 | 2 | Q8MJD1 | Q8mjd1 canis famil |
| 3 | 873.5 | 76.5 | 265 | 2 | Q61515 | Q61515 mus musculu |
| 4 | 686 | 60.1 | 258 | 2 | Q9GME1 | Q9gme1 ornithorhyn |
| 5 | 659 | 57.7 | 207 | 2 | Q9Z284 | Q9z284 mus musculu |
| 6 | 657 | 57.5 | 145 | 2 | Q6LDP5 | Q6ldp5 homo sapien |
| 7 | 648.5 | 56.8 | 254 | 2 | Q8K597 | Q8k597 rattus norv |
| 8 | 647.5 | 56.7 | 256 | 1 | PRN3_HUMAN | P24158 homo sapien |
| 9 | 646.5 | 56.6 | 237 | 2 | Q6LBN2 | Q6lbn2 homo sapien |
| 10 | 644.5 | 56.4 | 254 | 1 | PRN3_MOUSE | Q61096 mus musculu |
| 11 | 584.5 | 51.2 | 245 | 2 | Q6DFI0 | Q6df10 xenopus tro |
| 12 | 489 | 42.8 | 251 | 1 | CAP7_HUMAN | P20160 homo sapien |
| 13 | 437 | 38.3 | 219 | 1 | CAP7_PIG | P80015 sus scrofa |
| 14 | 392 | 34.3 | 264 | 2 | Q8QGF6 | Q8qgf6 xenopus lae |
| 15 | 392 | 34.3 | 264 | 2 | Q6GPY5 | Q6gpy5 xenopus lae |
| 16 | 387 | 33.9 | 258 | 2 | Q867B0 | Q867b0 canis famil |
| 17 | 384 | 33.6 | 265 | 2 | Q66KR6 | Q66kr6 xenopus lae |
| 18 | 381 | 33.4 | 283 | 2 | Q6UWY2 | Q6uwy2 homo sapien |
| 19 | 379 | 33.2 | 265 | 2 | Q7SYY8 | Q7syy8 xenopus lae |
| 20 | 379 | 33.2 | 266 | 1 | EL1_PIG | P00772 sus scrofa |
| 21 | 370.5 | 32.4 | 268 | 2 | Q9W7Q2 | Q9w7q2 paralichthy |
| 22 | 370 | 32.4 | 258 | 2 | Q6ISM6 | Q6ism6 homo sapien |
| 23 | 369 | 32.3 | 258 | 1 | EL1_HUMAN | Q9uni1 homo sapien |
| 24 | 366 | 32.0 | 266 | 2 | Q91X79 | Q91x79 mus musculu |
| 25 | 365 | 32.0 | 266 | 1 | EL1_RAT | P00773 rattus norv |
| 26 | 364 | 31.9 | 265 | 2 | Q6GNG0 | Q6gng0 xenopus lae |
| 27 | 364 | 31.9 | 266 | 2 | Q9D936 | Q9d936 mus musculu |
| 28 | 360.5 | 31.6 | 249 | 2 | Q9W7Q1 | Q9w7q1 paralichthy |
| 29 | 358 | 31.3 | 245 | 1 | MCT1_SHEEP | P80931 ovis aries |
| 30 | 358 | 31.3 | 266 | 2 | O46644 | O46644 macaca fasc |
| 31 | 357 | 31.3 | 266 | 1 | EL1_BOVIN | Q28153 bos taurus |
| 32 | 355 | 31.1 | 226 | 2 | Q86SR2 | Q86sr2 homo sapien |
| 33 | 355 | 31.1 | 251 | 2 | Q9GLN2 | Q9gln2 bos taurus |
| 34 | 354 | 31.0 | 278 | 2 | Q68FN6 | Q68fn6 brachydanio |
| 35 | 353.5 | 31.0 | 261 | 1 | CATG_MOUSE | P28293 mus musculu |
| 36 | 351.5 | 30.8 | 262 | 1 | GRAA_HUMAN | P12544 homo sapien |
| 37 | 351.5 | 30.8 | 266 | 2 | Q6AZC0 | Q6azc0 brachydanio |
| 38 | 350.5 | 30.7 | 267 | 2 | Q6DGW4 | Q6dgw4 brachydanio |
| 39 | 349.5 | 30.6 | 226 | 1 | DDN1_BOVIN | P80219 bos taurus |
| 40 | 349 | 30.6 | 260 | 2 | Q7SZC3 | Q7szc3 gallus gall |
| 41 | 348.5 | 30.5 | 236 | 2 | Q7SIG3 | Q7sig3 salmo salar |
| 42 | 346.5 | 30.3 | 255 | 1 | CATG_HUMAN | P08311 homo sapien |
| 43 | 342 | 29.9 | 269 | 2 | Q6AZF9 | Q6azf9 xenopus lae |
| 44 | 340.5 | 29.8 | 257 | 1 | GRAM_HUMAN | P51124 homo sapien |
| 45 | 339.5 | 29.7 | 260 | 1 | GRAA_MOUSE | P11032 mus musculu |

ALIGNMENTS

RESULT 1
ELNE_HUMAN
ID ELNE_HUMAN STANDARD; PRT; 267 AA.
AC P08246; P09649;
DT 01-AUG-1988 (Rel. 08, Created)
DT 01-AUG-1988 (Rel. 08, Last sequence update)
DT 25-OCT-2004 (Rel. 45, Last annotation update)
DE Leukocyte elastase precursor (EC 3.4.21.37) (Neutrophil elastase) (PMN
DE elastase) (Bone marrow serine protease) (Medullasin).
GN Name=ELA2;
OS Homo sapiens (Human).
OC Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX NCBI_TaxID=9606;
RN [1]
RP SEQUENCE FROM N.A.
RX MEDLINE=89374820; PubMed=2775493;
RA Farley D., Travis J., Salvesen G.;
RT "The human neutrophil elastase gene. Analysis of the nucleotide
RT sequence reveals three distinct classes of repetitive DNA.";
RL Biol. Chem. Hoppe-Seyler 370:737-744(1989).
RN [2]
RP SEQUENCE FROM N.A.
RX MEDLINE=88067782; PubMed=3479752;
RA Nakamura H., Okano K., Aoki Y., Shimizu H., Naruto M.;
RT "Nucleotide sequence of human bone marrow serine protease (medullasin)
RT gene.";
RL Nucleic Acids Res. 15:9601-9601(1987).
RN [3]
RP SEQUENCE FROM N.A.
RX MEDLINE=89008342; PubMed=2902087;
RA Takahashi H., Nukiwa T., Yoshimura K., Quick C.D., States D.J.,
RA Holmes M.D., Whang-Peng J., Knutsen T., Crystal R.G.;
RT "Structure of the human neutrophil elastase gene.";
RL J. Biol. Chem. 263:14739-14747(1988).
RN [4]
RP SEQUENCE FROM N.A.
RX MEDLINE=90211319; PubMed=2322278;
RA Okano K., Aoki Y., Shimizu H., Naruto M.;
RT "Functional expression of human leukocyte elastase (HLE)/medullasin in
RT eukaryotic cells.";
RL Biochem. Biophys. Res. Commun. 167:1326-1332(1990).
RN [5]
RP SEQUENCE FROM N.A., AND VARIANTS ILE-219; LEU-257 AND LEU-262.
RA Livingston R.J., Rieder M.J., Chung M.-W., Ritchie T.K., Olson A.N.,
RA Nguyen C.P., Nguyen D.A., Poel C.L., Robertson P.D., Schackwitz W.S.,
RA Sherwood J.K., Sherwood A.M., Leithauser B.J., Nickerson D.A.;
RT "NIEHS-SNPs, environmental genome project, NIEHS ES15478, Department
RT of Genome Sciences, Seattle, WA (URL: http://egp.gs.washington.edu).";
RL Submitted (APR-2004) to the EMBL/GenBank/DDBJ databases.
RN [6]
RP SEQUENCE OF 30-267 FROM N.A.
RX MEDLINE=88032918; PubMed=2822677;
RA Okano K., Aoki Y., Sakurai T., Kajitani M., Kanai S., Shimazu T.,

```
RA   Shimizu H., Naruto M.;
RT   "Molecular cloning of complementary DNA for human medullasin: an
RT   inflammatory serine protease in bone marrow cells.";
RL   J. Biochem. 102:13-16(1987).
RN   [7]
RP   SEQUENCE OF 75-267 FROM N.A.
RX   MEDLINE=88115408; PubMed=3422232;
RA   Takahashi H., Nukiwa T., Basset P., Cystal R.G.;
RT   "Myelomonocytic cell lineage expression of the neutrophil elastase
RT   gene.";
RL   J. Biol. Chem. 263:2543-2547(1988).
RN   [8]
RP   SEQUENCE OF 30-247.
RX   MEDLINE=87175647; PubMed=3550808;
RA   Sinha S., Watorek W., Karr S., Giles J., Bode W., Travis J.;
RT   "Primary structure of human neutrophil elastase.";
RL   Proc. Natl. Acad. Sci. U.S.A. 84:2228-2232(1987).
RN   [9]
RP   SEQUENCE OF 262-267.
RX   MEDLINE=91315473; PubMed=1859409;
RA   Aoki Y., Hase T.;
RT   "The primary structure and elastinolytic activity of medullasin (a
RT   serine protease of bone marrow).";
RL   Biochem. Biophys. Res. Commun. 178:501-506(1991).
RN   [10]
RP   PRELIMINARY SEQUENCE OF 30-103.
RA   Travis J., Giles P.J., Porcelli L., Reilly C.F., Baugh R., Powers J.;
RL   (In) Protein degradation in health and disease, Ciba Foundation
RL   Symposium, pp.75:51-68, Excerpta Medica, Amsterdam and Oxford (1980).
RN   [11]
RP   SEQUENCE OF 30-49.
RX   MEDLINE=89315847; PubMed=2501794;
RA   Gabay J.E., Scott R.W., Campanelli D., Griffith J., Wilde C.,
RA   Marra M.N., Seeger M., Nathan C.F.;
RT   "Antibiotic proteins of human polymorphonuclear leukocytes.";
RL   Proc. Natl. Acad. Sci. U.S.A. 86:5610-5614(1989).
RN   [12]
RP   X-RAY CRYSTALLOGRAPHY (1.84 ANGSTROMS).
RX   MEDLINE=89098932; PubMed=2911584;
RA   Navia M.A., McKeever B.M., Springer J.P., Lin T.-Y., Williams H.R.,
RA   Fluder E.M., Dorn C.P., Hoogsteen K.;
RT   "Structure of human neutrophil elastase in complex with a peptide
RT   chloromethyl ketone inhibitor at 1.84-A resolution.";
RL   Proc. Natl. Acad. Sci. U.S.A. 86:7-11(1989).
RN   [13]
RP   X-RAY CRYSTALLOGRAPHY (2.3 ANGSTROMS).
RX   MEDLINE=88271660; PubMed=3391280; DOI=10.1016/0014-5793(88)80118-2;
RA   Wei A.-Z., Mayr I., Bode W.;
RT   "The refined 2.3-A crystal structure of human leukocyte elastase in a
RT   complex with a valine chloromethyl ketone inhibitor.";
RL   FEBS Lett. 234:367-373(1988).
RN   [14]
RP   X-RAY CRYSTALLOGRAPHY (1.7 ANGSTROMS).
RX   MEDLINE=87053808; PubMed=3640709;
RA   Bode W., Wei A.-Z., Huber R., Meyer E., Travis J., Neumann S.;
RT   "X-ray crystal structure of the complex of human leukocyte elastase
RT   (PMN elastase) and the third domain of the turkey ovomucoid
RT   inhibitor.";
RL   EMBO J. 5:2453-2458(1986).
RN   [15]
RP   VARIANTS CH VAL-32; PHE-177 AND GLN-191.
RX   MEDLINE=20047772; PubMed=10581030; DOI=10.1038/70544;
RA   Horwitz M., Benson K.F., Person R.E., Aprikyan A.G., Dale D.C.;
RT   "Mutations in ELA2, encoding neutrophil elastase, define a 21-day
RT   biological clock in cyclic haematopoiesis.";
RL   Nat. Genet. 23:433-436(1999).
CC   -!- FUNCTION: Medullasin modifies the functions of natural killer
CC       cells, monocytes and granulocytes.
CC   -!- CATALYTIC ACTIVITY: Hydrolysis of proteins, including elastin.
CC       Preferential cleavage: Val-|-Xaa > Ala-|-Xaa.
CC   -!- TISSUE SPECIFICITY: Bone marrow cells.
CC   -!- DISEASE: Defects in ELA2 are a cause of cyclic haematopoiesis (CH)
CC       [MIM:162800]; also known as cyclic neutropenia. CH is an autosomal
CC       dominant disease in which blood-cell production from the bone
CC       marrow oscillates with 21-day periodicity. Circulating neutrophils
CC       vary between almost normal numbers and zero. During intervals of
CC       neutropenia, affected individuals are at risk for opportunistic
CC       infection. Monocytes, platelets, lymphocytes and reticulocytes
CC       also cycle with the same frequency.
CC   -!- SIMILARITY: Belongs to the peptidase S1 family. Elastase
CC       subfamily.
CC   ---------------------------------------------------------------------
CC   This SWISS-PROT entry is copyright. It is produced through a collaboration
CC   between the Swiss Institute of Bioinformatics and the EMBL outstation -
CC   the European Bioinformatics Institute. There are no restrictions on its
CC   use by non-profit institutions as long as its content is in no way
CC   modified and this statement is not removed. Usage by and for commercial
CC   entities requires a license agreement (See http://www.isb-sib.ch/announce/
CC   or send an email to license@isb-sib.ch).
CC   ---------------------------------------------------------------------
DR   EMBL; J03545; AAA52378.1; -.
DR   EMBL; Y00477; CAA68537.1; -.
DR   EMBL; X05875; CAA29299.1; -.
DR   EMBL; X05875; CAA29300.1; ALT_INIT.
DR   EMBL; M20203; AAA36359.1; -.
DR   EMBL; M20199; AAA36359.1; JOINED.
DR   EMBL; M20200; AAA36359.1; JOINED.
DR   EMBL; M20201; AAA36359.1; JOINED.
DR   EMBL; AY596461; AAS89303.1; -.
DR   EMBL; M34379; AAA36173.1; -.
DR   EMBL; D00187; BAA00128.1; -.
DR   PIR; A31976; ELHUL.
DR   PDB; 1B0F; X-ray; A=30-247.
DR   PDB; 1H1B; X-ray; A/B=30-247.
DR   PDB; 1HNE; X-ray; E=30-247.
DR   PDB; 1PPF; X-ray; E=30-247.
DR   PDB; 1PPG; X-ray; E=30-247.
DR   MEROPS; S01.131; -.
DR   Genew; HGNC:3309; ELA2.
DR   MIM; 130130; -.
DR   MIM; 162800; -.
DR   GO; GO:0004234; F:macrophage elastase activity; TAS.
DR   InterPro; IPR009003; Pept_Ser_Cys.
DR   InterPro; IPR001254; Peptidase_S1.
DR   InterPro; IPR001314; Peptidase_S1A.
DR   Pfam; PF00089; Trypsin; 1.
DR   PRINTS; PR00722; CHYMOTRYPSIN.
DR   PROSITE; PS50240; TRYPSIN_DOM; 1.
DR   PROSITE; PS00134; TRYPSIN_HIS; 1.
DR   PROSITE; PS00135; TRYPSIN_SER; 1.
KW   3D-structure; Direct protein sequencing; Disease mutation;
KW   Glycoprotein; Hydrolase; Polymorphism; Serine protease; Signal.
FT   SIGNAL       1     27       Potential.
FT   PROPEP      28     29
FT   CHAIN       30    267       Leukocyte elastase.
FT   ACT_SITE    70     70       Charge relay system.
FT   ACT_SITE   117    117       Charge relay system.
FT   ACT_SITE   202    202       Charge relay system.
FT   DISULFID    55     71
FT   DISULFID   151    208
FT   DISULFID   181    187
FT   DISULFID   198    223
FT   CARBOHYD    88     88       N-linked (GlcNAc. . .) (Potential).
FT   CARBOHYD   124    124       N-linked (GlcNAc. . .).
FT   CARBOHYD   173    173       N-linked (GlcNAc. . .).
FT   VARIANT     32     32       G -> V (in CH).
FT                               /FTId=VAR_009538.
FT   VARIANT    177    177       V -> F (in CH).
FT                               /FTId=VAR_009539.
FT   VARIANT    191    191       R -> Q (in CH).
FT                               /FTId=VAR_009540.
FT   VARIANT    219    219       V -> I.
FT                               /FTId=VAR_019237.
FT   VARIANT    257    257       P -> L.
FT                               /FTId=VAR_019238.
FT   VARIANT    262    262       P -> L.
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FT                              /FTId=VAR_019239.
FT   CONFLICT    107    107     N -> D (in Ref. 7).

  Query Match             100.0%;  Score 1142;  DB 1;  Length 267;
  Best Local Similarity   100.0%;  Pred. No. 6.6e-96;
  Matches  219;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 IVGGRRARPHAWPFMVSLQLRGGHFCGATLIAPNFVMSAAHCVANVNVRAVRVVLGAHNL 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         30 IVGGRRARPHAWPFMVSLQLRGGHFCGATLIAPNFVMSAAHCVANVNVRAVRVVLGAHNL 89

Qy         61 SRREPTRQVFAVQRIFENGYDPVNLLNDIVILQLNGSATINANVQVAQLPAQGRRLGNGV 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         90 SRREPTRQVFAVQRIFENGYDPVNLLNDIVILQLNGSATINANVQVAQLPAQGRRLGNGV 149

Qy        121 QCLAMGWGLLGRNRGIASVLQELNVTVVTSLCRRSNVCTLVRGRQAGVCFGDSGSPLVCN 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        150 QCLAMGWGLLGRNRGIASVLQELNVTVVTSLCRRSNVCTLVRGRQAGVCFGDSGSPLVCN 209

Qy        181 GLIHGIASFVRGGCASGLYPDAFAPVAQFVNWIDSIIQR 219
              |||||||||||||||||||||||||||||||||||||||
Db        210 GLIHGIASFVRGGCASGLYPDAFAPVAQFVNWIDSIIQR 248


RESULT 2
Q8MJD1
ID   Q8MJD1      PRELIMINARY;      PRT;   282 AA.
AC   Q8MJD1;
DT   01-OCT-2002 (TrEMBLrel. 22, Created)
DT   01-OCT-2002 (TrEMBLrel. 22, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Neutrophil elastase.
GN   Name=ELA2;
OS   Canis familiaris (Dog).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Carnivora; Fissipedia; Canidae; Canis.
OX   NCBI_TaxID=9615;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=22375507; PubMed=12487025;
RA   Katen L.J., Aprikyan A.G., Dale D.C., Osborne W.R.A.;
RT   "Molecular Cloning and Sequencing of the Canine Neutrophil Elastase
RT   cDNA.";
RL   DNA Seq. 13:221-223(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RA   Benson K.F., Albani D., Person R.E., Horwitz M.;
RL   Submitted (JAN-2003) to the EMBL/GenBank/DDBJ databases.
CC   -!- SIMILARITY: Belongs to peptidase family S1.
DR   EMBL; AF494190; AAM95916.1; -.
DR   EMBL; AY221639; AAO65978.1; -.
DR   PIR; A60551; A60551.
DR   HSSP; P08246; 1PPF.
DR   GO; GO:0004263; F:chymotrypsin activity; IEA.
DR   GO; GO:0008233; F:peptidase activity; IEA.
DR   GO; GO:0004295; F:trypsin activity; IEA.
DR   GO; GO:0006508; P:proteolysis and peptidolysis; IEA.
DR   InterPro; IPR001254; Peptidase_S1.
DR   InterPro; IPR001314; Peptidase_S1A.
DR   InterPro; IPR009003; Pept_Ser_Cys.
DR   Pfam; PF00089; Trypsin; 1.
DR   PRINTS; PR00722; CHYMOTRYPSIN.
DR   SMART; SM00020; Tryp_SPc; 1.
DR   PROSITE; PS50240; TRYPSIN_DOM; 1.
DR   PROSITE; PS00134; TRYPSIN_HIS; UNKNOWN_1.
DR   PROSITE; PS00135; TRYPSIN_SER; 1.
KW   Hydrolase; Protease; Serine protease.
SQ   SEQUENCE   282 AA;  29920 MW;  B6F31953BFA4F9B3 CRC64;

  Query Match             79.2%;  Score 905;  DB 2;  Length 282;
  Best Local Similarity   77.6%;  Pred. No. 2.9e-74;
  Matches  170;  Conservative   22;  Mismatches   27;  Indels    0;  Gaps    0;

Qy          1 IVGGRRARPHAWPFMVSLQLRGGHFCGATLIAPNFVMSAAHCVANVNVRAVRVVLGAHNL 60
              ||||| |:|||||||||||| |||||||  |||||||||||||||  :| |:| |||||:|
Db         31 IVGGRPAQPHAWPFMVSLQRRGGHFCGGTLIAPNFVMSAAHCVDGLNFRSVVVVLGAHDL 90

Qy         61 SRREPTRQVFAVQRIFENGYDPVNLLNDIVILQLNGSATINANVQVAQLPAQGRRLGNGV 120
               || |||:|||||:||||:||| |:||||:|||||||||||||||:|||| : :||||
Db         91 GERESTRQLFAVQRVFENGFDPVRLVNDIVLLQLNGSATINANVQVARLPAQNQGVGNGV 150

Qy        121 QCLAMGWGLLGRNRGIASVLQELNVTVVTSLCRRSNVCTLVRGRQAGVCFGDSGSPLVCN 180
              |||||||| ||  :    :|||||||||:|||||||||||  |:||:|||||| |||||
Db        151 QCLAMGWGQLGTAQPPPRILQELNVTVVTTLCRRSNVCTLVPRRRAGICFGDSGGPLVCN 210

Qy        181 GLIHGIASFVRGGCASGLYPDAFAPVAQFVNWIDSIIQR 219
              ||| ||.||:|| |||| :|||||||||||:||:|||:|
Db        211 GLIQGIDSFIRGSCASGFFPDAFAPVAQFVDWINSIIRR 249


RESULT 3
Q61515
ID   Q61515      PRELIMINARY;      PRT;   265 AA.
AC   Q61515;
DT   01-NOV-1996 (TrEMBLrel. 01, Created)
DT   01-NOV-1996 (TrEMBLrel. 01, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Neutrophil elastase.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   STRAIN=BALB/c;
RX   MEDLINE=94309676; PubMed=8035830;
RA   Nuchprayoon I., Meyers S., Scott L.M., Suzow J., Hiebert S.,.
RA   Friedman A.D.;
RT   "PEBP2/CBF, the murine homolog of the human myeloid AML1 and PEBP2
RT   beta/CBF beta proto-oncoproteins, regulates the murine myeloperoxidase
RT   and neutrophil elastase genes in immature myeloid cells.";
RL   Mol. Cell. Biol. 14:5558-5568(1994).
CC   -!- SIMILARITY: Belongs to peptidase family S1.
DR   EMBL; U04962; AAB60670.1; -.
DR   EMBL; U06076; AAB60670.1; JOINED.
DR   PIR; I48679; I48679.
DR   HSSP; P08246; 1PPF.
DR   MEROPS; S01.131; -.
DR   GO; GO:0004263; F:chymotrypsin activity; IEA.
DR   GO; GO:0008233; F:peptidase activity; IEA.
DR   GO; GO:0004295; F:trypsin activity; IEA.
DR   GO; GO:0006508; P:proteolysis and peptidolysis; IEA.
DR   InterPro; IPR001254; Peptidase_S1.
DR   InterPro; IPR001314; Peptidase_S1A.
DR   InterPro; IPR009003; Pept_Ser_Cys.
DR   Pfam; PF00089; Trypsin; 1.
DR   PRINTS; PR00722; CHYMOTRYPSIN.
DR   SMART; SM00020; Tryp_SPc; 1.
DR   PROSITE; PS50240; TRYPSIN_DOM; 1.
DR   PROSITE; PS00135; TRYPSIN_SER; 1.
KW   Hydrolase; Protease; Serine protease.
SQ   SEQUENCE   265 AA;  28654 MW;  8744D5CE3A72E09D CRC64;

  Query Match             76.5%;  Score 873.5;  DB 2;  Length 265;
  Best Local Similarity   75.9%;  Pred. No. 2e-71;
  Matches  167;  Conservative   20;  Mismatches   32;  Indels    1;  Gaps    1;

Qy          1 IVGGRRARPHAWPFMVSLQLRGGHFCGATLIAPNFVMSAAHCVANVNVRAVRVVLGAHNL 60
              ||||| ||||||||| ||| |||||||||||| |||||| |||  :| |:|:|||||||:|
Db         29 IVGGRPARPHAWPFMASLQRRGGHFCGATLIARNFVMSAVHCVNGLNFRSVQVVLGAHDL 88

Qy         61 SRREPTRQVFAVQRIFENGYDPVNLLNDIVILQLNGSATINANVQVAQLPAQGRRLGNGV 120
               |:| ||| |:|| |||||:||  |||||||:||||||||||||||||||: :|:
Db         89 RRQERTRQTFSVQGIFENGFDPSQLLNDIVIIQLNGSATINANVQVAQLPAQGQGVGDRT 148

Qy        121 QCLAMGWGLLGRNRGIASVLQELNVTVVTSLC-RRSNVCTLVRGRQAGVCFGDSGSPLVC 179
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